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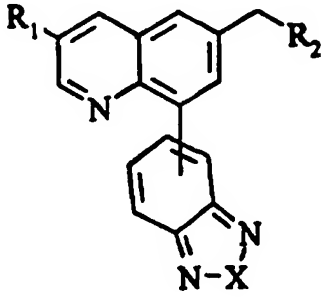
(54) Title: QUINOLINE DERIVATIVES AS TYPE IV PHOSPHODIESTERASE INHIBITORS

(57) Abstract

8-(Benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinolines, in free or N-oxide or acid addition or ammonium salt form, are novel and found to be useful as pharmaceuticals, e.g., as PDEIV inhibitors, for example in the treatment of asthma. Novel methods of treatment using the compounds, processes for making the compounds, and pharmaceutical compositions comprising the compounds are also provided. For example, compounds of formula (1), wherein X is -O-, -S-, or $-N(CH_3)-$, R_1 is hydrogen or (C_{1-4})alkyl, and R_2 is (C_{1-4})alkyl or pyridyl, in free or N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.



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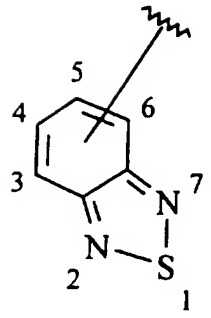
QUINOLINE DERIVATIVES AS TYPE IV PHOSPHODIESTERASE INHIBITORS

The present invention relates to novel quinoline derivatives, processes for their production, their use as pharmaceuticals and pharmaceutical compositions containing them.

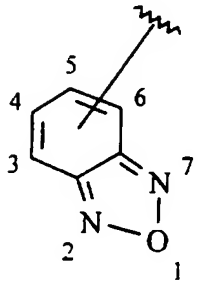
It has now surprisingly been discovered that quinoline derivatives substituted at the 8-position with a benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl or 2-methyl-2H-benzo[*d*]triazolyl moiety (which form a completely new class of compounds) have highly desirable and useful properties as hereinafter described, in particular good oral activity, improved side effect profile and potent selective inhibition of PDE IV.

Thus, in a broadest aspect, the present invention provides an 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline, in free, N-oxide, or acid addition or ammonium salt form. The 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline is optionally substituted on the quinoline nucleus, e.g., at the 3- and/or 6-positions. Suitable substituents at the 3-position include C_{1-4} alkyl, e.g., methyl, to form a 3-(C_{1-4}alkyl)-8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline. Substituents at the 6-position are preferably linked via a carbon atom, i.e., to provide a 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-6-carbo-quinoline or a 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-3-(C_{1-4}alkyl)-6-carbo-quinoline. Particularly preferred at the 6-position is pyridylmethyl, e.g., pyridin-4-yl-methyl. Preferred compounds thus include, for example, 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-6-(pyridylmethyl)-quinolines or 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-3-(C_{1-4} alkyl)-(6-pyridylmethyl)-quinolines, in free, N-oxide, or acid addition or ammonium salt form.

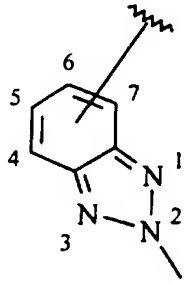
By benzo[*c*]thiadiazolyl is meant a radical of the following structure:



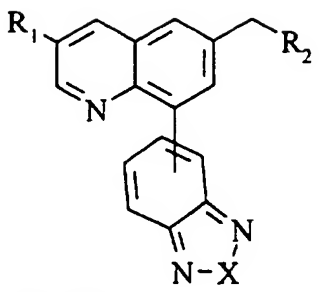
By benzo[*c*]furazanyl is meant a radical of the following structure:



By 2-methyl-2H-benzo[*d*]triazolyl is meant a radical of the following structure:



The 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline of the invention is preferably a compound of formula I:



Formula I

wherein

X is -O-, -S-, or -N(CH_3)- (preferably -O- or -S-),

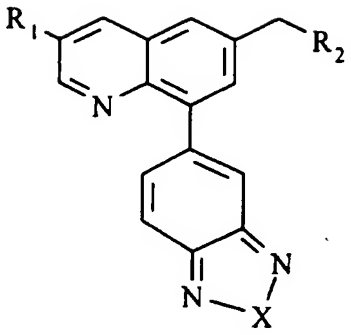
R_1 is hydrogen or (C_{1-4})alkyl (preferably methyl or hydrogen), and

R_2 is (C_{1-4})alkyl or pyridyl (preferably 4-pyridyl)

in free or N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

For example, the invention includes compounds of formula I wherein X is O or S, R_1 is hydrogen or (C_{1-4})alkyl, and R_2 is (C_{1-4})alkyl or pyridyl, in free base or acid addition salt form.

Suitably, the 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[d]triazolyl)-quinoline of the invention is 8-(benzo[*c*]thiadiazol-3- or -4-yl)- or 8-(benzo[*c*]furazan-3- or -4-yl)- or 8-(2-methyl-2H-benzo[*d*]triazol-4- or -5-yl)- -quinoline, preferably 8-(benzo[*c*]thiadiazol-4-yl)- or 8-(benzo[*c*]furazan-4-yl)- or 8-(2-methyl-2H-benzo[*d*]triazol-5-yl)-quinoline e.g., a compound of formula Ia:



Formula Ia

wherein X, R_1 and R_2 are as defined above, in free, N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

Alkyl groups may be branched or straight chain, preferably straight chain, methyl being preferred.

The compounds may be in free form or may be associated with an acid to form an acid addition salt or with an alkyl halide to form an ammonium salt, or N-oxidated, and these different forms of the molecule are considered to be pharmaceutically useful. Suitable pharmaceutically acceptable acid addition salts for use in accordance with the present invention include salts of inorganic acids, for example the hydrochloride from hydrochloric acid, and salts of organic acids, for example the hydrogen maleinate from maleic acid or the oxalate from oxalic acid. Ammonium and N-oxide forms are provided particularly when R_2 is pyridyl such that the nitrogen in the pyridyl ring is alkylated or oxidized, e.g. so that ammonium salts include N-alkylated (e.g., N-methylated) pyridinium salts and N-oxides include compounds of formula I or Ia wherein R_2 is N-oxo-pyridyl, e.g., 1-oxo-pyridin-4-yl.

In a further aspect the present invention provides a process for the production of an 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline, comprising reacting

(i) a 3-Q- or 4-Q- -benzo[*c*]thiadiazole, -benzo[*c*]furazan, or -2-methyl-2H-benzo[*d*]triazole with an 8-Q'-quinoline (e.g., optionally 3-(C_{1-4}alkyl)- and/or 6-carbo- -substituted)

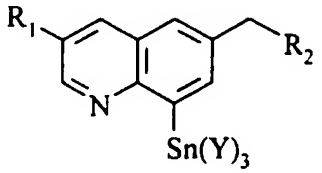
wherein Q and Q' are leaving groups capable of participating in a cross-coupling reaction, (for example when one of Q and Q' is halogen (e.g., bromine) or trifluoromethanesulfonyl, the other is suitably a metallic or organometallic leaving group (e.g., $B(OH)_2$-, $(CH_3(CH_2)_3)_3Sn$-, Li-, ClZn-, or BrMg-),

(ii) optionally N-alkylating (e.g. reacting with an alkyl halide, e.g., MeI) or N-ozidizing (e.g., reacting with a peroxide reagent, for example m-chloro-perbenzoic acid) the product,

and recovering the compound thus obtained, in free, N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

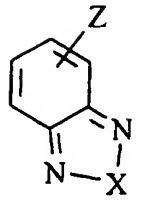
The reaction is suitably carried out in the presence of a palladium or nickel catalyst with or without the addition of further metals such as copper, for example in analogy to known aryl coupling reactions, e.g. a Stille, Suzuki, Negishi or Heck reaction, for example as described in Example 1. Recovery and purification of the 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline thus obtained may be accomplished using standard procedures, e.g., by chromatography or crystallization.

For example, compounds of formula I or Ia are suitably prepared by reacting a quinoline of formula II



II

wherein R_1 and R_2 are as defined above and Y is (C_{1-4})alkyl, with a benzo[*c*]thiadiazole, benzo[*c*]furazan, or 2-methyl-2H-benzo[*d*]triazole of formula III

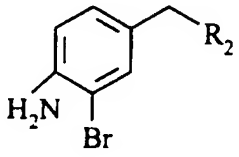


III

wherein X is as defined above and Z is bromine, iodine or SO_3CF_3, and recovering the compound thus obtained in free base form or in form of an acid addition salt.

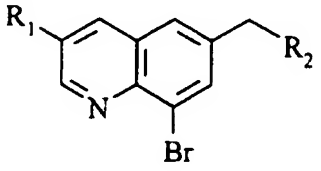
In the compounds of formula II, Y is preferably butyl, e.g., n-butyl. In the compounds of formula III, Z is preferably bromine.

The starting compounds of formula II may be produced from the compounds of formula IV



IV

wherein R_2 is as defined above, or the compounds of formula V



V

wherein R_1 and R_2 are as defined above, according to known methods. Thus the compounds of formula V can be obtained using 2-R_1-acrolein in the presence of an acid. If R_1 is hydrogen, acrolein can be prepared <u>in situ</u> (quinoline synthesis according to Skraup).

The compounds of formulae III and IV are known or may be produced by known methods. The compound of formula IV wherein R_2 is pyridin-4-yl, for example, may be prepared as described in the international patent application WO 94/22852.

EXAMPLES

The following examples illustrate the invention. The temperatures are given in degrees Celsius.

Example 1: 8-(benzo[*c*]furazan-4-yl)-6-(pyridin-4-yl-methyl)-quinoline

a) 8-bromo-6-(pyridin-4-yl-methyl)-quinoline

The compound is obtained by Skraup quinoline synthesis as described in Manske R.H.F., Organic Reactions, 7, 59 (1953), using 2-bromo-4-(pyridin-4-yl-methyl)-phenylamine (compound of formula IV). Rf (Silica gel, hexane - ethyl acetate - ethanol 65:35:10): 0.25; melting point: 96-99°C.

b) 6-(pyridin-4-yl-methyl)-8-tributylstannyl-quinoline

8-bromo-6-(pyridin-4-yl-methyl)-quinoline (2.5 g, 8.36 mmol), hexabutyldistannane (5.33 g, 9.2 mmol) and tetrakis(triphenylphosphine)palladium (485 mg, 0.42 mmol) are suspended in toluene (40 ml) and heated to reflux under argon for 24 hours, after which time the reaction mixture is allowed to cool to room temperature and filtered. The filtrate is diluted with ethyl acetate, washed with aq. Na_2CO_3, dried (Na_2SO_4) and evaporated to give a yellow oil which is subjected to chromatography (silica gel, hexane - ethyl acetate 2:1) to give the title product as a yellow oil. Rf (silica gel, hexane - ethyl acetate 2:1): 0.18. MS (FAB): 510 (MH+).

c) 8-(benzo[*c*]furazan-4-yl)-6-(pyridin-4-yl-methyl)-quinoline

A suspension of 4-bromo-benzo[c]furazan (420 mg, 2.1 mmol), 8-tributylstannyl-6-(pyridin-4-yl-methyl)-quinoline (1.0 g, 1.96 mmol) and tetrakis(triphenylphosphine)palladium (20 mg, 0.017 mmol) in DMF (15 ml) is heated to reflux for 8 hours, after which the mixture is allowed to cool, diluted with ethyl acetate and extracted with aq. HCl. The combined aqueous extracts are made alkaline with aq. Na_2CO_3, and extracted with ethyl acetate. The organic extracts are dried (Na_2SO_4) to give a brown oil from which the title compound crystallizes. Melting point 154-157°C; MS (FAB): 339 (MH+).

Example 2: 8-(benzo[*c*]thiadiazol-4-yl)-6-(pyridin-4-yl-methyl)-quinoline

Obtained in analogous manner to Example 1c), but using 4-bromo-benzo[c]thiadiazole. The hydrochloride has a melting point of 150-155°C. MS (FAB): 391(MH+).

Example 3: 6-(pyridin-4-yl-methyl)-8-(benzo[*c*]furazan-3-yl)-quinoline

Obtained in analogous manner to Example 1c), but using 3-bromo-benzo[c]furazan. Melting point: 183-188°. MS (FAB): 339(MH+).

Example 4: 8-(benzo[*c*]furazan-4-yl)-3-methyl-6-(pyridin-4-yl-methyl)-quinoline

a) 8-bromo-3-methyl-6-(pyridin-4-yl-methyl)-quinoline

A solution of 2-bromo-4-(pyridin-4-yl-methyl)-phenylamine (6.0 g, 22.8 mmol), p-toluenesulfonic acid (4.3 g, 22.8 mmol) and methacrolein (20.0 ml, 228 mmol) in toluene (200 ml), is heated to reflux in a Dean-Stark apparatus for 8 hours. The reaction mixture is allowed to cool overnight, and the toluene decanted. The resinous residue is stirred with CH_2Cl_2 (150 ml) and sat. aq. K_2CO_3 (80 ml) until dissolved. The organic phase is separated and washed with brine (50 ml); the aqueous phase is extracted with CH_2Cl_2 (2 x 50 ml). The combined organic extracts are dried ($MgSO_4$) and evaporated to yield a brown oil. The product is isolated by

chromatography (silica gel, hexane-ethyl acetate-ethanol 65:35:10, Rf 0.25) as a crystalline solid, melting point 108-109°. MS(CI, C_2H_5): 313, 315 (100, MH+).

b) 3-Methyl-6-(pyridin-4-yl-methyl)-8-tributylstannyl-quinoline

Prepared from 3-methyl-6-(pyridin-4-yl-methyl)-8-bromo-quinoline in analogous fashion to Example 1b). Yellow oil, Rf (silica gel, hexane-ethyl acetate-ethanol 65:35:10) 0.5; MS(FAB): 523 (M+).

c) 8-(benzo[c]furazan-4-yl)-3-methyl-6-(pyridin-4-yl-methyl)-quinoline

Obtained from 3-methyl-6-(pyridin-4-yl-methyl)-8-tributylstannyl-quinoline and 4-bromo-benzo[c]furazan in analogous fashion to step 1c). Rf (silica gel, hexane-ethyl acetate-ethanol 65:35:10) 0.25; MS (EI) 352 (MH+); melting point 193-194°C.

Example 5: 8-(benzo[*c*]thiadiazol-4-yl)-3-methyl-6-(pyridin-4-yl-methyl)-quinoline

Obtained in an analogous manner to example 4, using 4-bromo-benzo[c]thiadiazole. MS (EI) 367 (100, (M-H)+), 276. The hydrogen maleinate has a melting point of 147-148°C.

Example 6: 8-(2-methyl-2H-benzo[*d*]triazol-5-yl)-6-(pyridin-4-ylmethyl)-quinoline

a) 5-bromo-2-methyl-2H-benzotriazole (Sandmeyer reaction)

5-amino-2-methyl-2H-benzo[d]triazole (Eur. J. Med. Chem. 1992, 161) (1.64 g,11 mmol) is treated with NaNO2 (830 mg,12 mmol) in 30 ml conc. sulfuric acid at 10° C. After 30 min. the solution is added to CuBr (1.66g,11.6 mmol) in 9ml 48% HBr and 9 ml water at 5°C, followed by heating at 90°C for 15 min. After extraction with ethyl acetate the crude oil is flashed over silica (hexanes: ethylacetate 2:1). 5-bromo-2-methyl-2H-benzo[d]triazole crysatallised as

white-yellow needles, having a melting point of 51-54°C. H-NMR (d-DMSO): 8.25(s, 1H), 7.93 (d,1H), 7.55 (d,1H), 4.50 (s, 3H).

b) 2-Methyl-2H-benzo[d]triazole-5-boronic acid

5-Bromo-2-methyl-2H-benzo[d]triazole (500 mg,2.4 mmol) and triisopropylborate (500mg,2.6 mmol) are dissolved in THF (20 ml) at -78°C and n-BuLi (1.6 ml in hexanes, 2.6 mmol) is added. The reaction mixture is stirred for 30 min at -78°C and then 3h at room temperature. The reaction is quenched with water (20 ml), acidified with 4N HCl and extracted with ethyl acetate (3x50ml). The organic phase is dried over sodium sulfate, filtered and evaporated to give the title compound as a white solid having a melting point >260°C, 1H-NMR (d-DMSO): 8.39 (s, 1H), 8.24 (s,2H), 7.88-7.75 (AB-system, 2H), 4.51 (s,3H).

c) 8-(2-methyl-2H-benzo[d]triazol-5-yl)-6-(pyridin-4-ylmethyl)-quinoline

2-Methyl-2H-benzo[d]triazole-5-boronic acid (240mg, 1.36 mmol) and 8-bromo-6-(4-methylpyridyl)quinoline (470 mg, 1.56 mmol) are kept at 90°C in 40 ml of toluene in the presence of Pd(OAc)2 (12 mg), P(o-tolyl)3 (17 mg), 2M Na2CO3 solution (1.8 ml) and ethanol (0.5 ml) for 20 h.The cold reaction mixture is filtered, diluted with brine (80 ml) and extracted with ethyl acetate (3x50ml), dried over sodium sulfate, filtered and evaporated. The crude product is purified over silica (ethyl acetate) yielding a yellowish oil. The melting point for the oxalate is 98-103°C. 1H-NMR(CDCl3,free base): 8.91 (dd,1H), 8.55 (d,2H), 8.18 (dd, 1H), 8.08 (s, 1H), 7.92 (d, 1H), 7.71 (dd,1H), 7.62 (s,2H), 7.42 (dd,1H), 7.20 (d, 2H), 4.53 (s, 3H), 4.20 (s, 2H); MS (EI) 351 (70, M+), 350 (100), 259 (64); Found: C, 65.17; H, 4.61; N, 15.84. C24H19N5O4 requires C, 65.29; H, 4.30; N,15.86.

8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinolines, e.g., of formula I or Ia, particularly the compounds of the examples above, in free, N-

oxide or pharmaceutically acceptable acid addition or ammonium salt form, hereinafter referred to as AGENTS OF THE INVENTION, exhibit pharmacological activity and are useful as pharmaceuticals, e.g. for therapy in the treatment of diseases and conditions as hereinafter set forth.

AGENTS OF THE INVENTION are selective inhibitors of the Type IV cAMP phosphodiesterase isoenzyme family, and have relatively little effect on the phosphodiesterase isoenzymes of Types I,II,III and V. As such AGENTS OF THE INVENTION can be used in conditions where elevation of cAMP levels through the selective inhibition of Type IV phosphodiesterase isoenzymes is useful. Since elevation of cAMP levels in inflammatory cells such as eosinophils inhibits their activation, AGENTS OF THE INVENTION are useful in inflammatory conditions in which eosinophil activation plays a role.

AGENTS OF THE INVENTION, by virtue of their inhibition of Type IV phosphodiesterase in human eosinophils, are useful in the treatment of atopic and non-atopic asthma, as supported by the models of PDE inhibition, inhibition of eosinophil activation and bronchodilator models described below.

PDE ISOENZYME INHIBITION

The utility of the compounds to selectively inhibit Type IV cAMP phosphodiesterase is demonstrated by the following test:

Human phosphodiesterase inhibition assay

All isoenzyme preparations are derived from human sources. Type III and IV preparations are obtained taking advantage of the predominance of type III isoenzymes in platelets and type IV isoenzymes in neutrophils applying the following techniques:

Citrated human blood is collected and neutrophils separated by dextran sedimentation, density gradient centrifugation on a mixture of Histopaque 1077 and 1119 with a final density of 1.089g/l and hypotonic lysis of erythrocytes. Human platelets from the same source are washed with PBS (NaCl 140 mM, KCl 2.7 mM, KH_2PO_4 1.5 mM, Na_2HPO_4 8.1 mM, pH 7.4). Neutrophils and platelets are suspended in 10ml of buffer (0.24 M sucrose, 1 mM EDTA, 1mM dithiothreitol, 10mM tris HCl, pH 7.4) containing the following protease inhibitor solutions: 5 µl/ml of phenylmethylsulphonylfluoride (7 mg/ml in 2-propanol), 1 µl/ml leupeptin and pepstatin A (1 mg/ml each, in ethanol). After sonication (15 sec at 4°C) using a probe sonicator, homogenates are centrifuged (2200g). The pellet is resuspended in 10ml of buffer and the sonication repeated. Pooled supernatants are stored at -20°C.

Other isoenzymes are partially purified employing chromatographic methods as described in the art (Types I and V from human lung, and type II from human platelets). PDE activity is assayed in the presence and absence of test substance at varying concentration using the ion-exchange column method described by Thompson et al., Nucleotide Res., 10, 69-92 (1979), with 1µM [^{3}H]-cyclic AMP as substrate (Types III and IV) or 0.5µM calcium, 0.125µM calmodulin and 1.0µM [^{3}H]-cyclic AMP (Type I) or 100µM[^{3}H]-cyclic AMP (Type II) or 1.0µM[^{3}H]-cyclic GMP (Type V).

In this test method, AGENTS OF THE INVENTION predominantly inhibit PDE isoenzymes of the type IV, having relatively little effect in relation to types I, II, III and V. Within the PDE IV isotype, the AGENTS OF THE INVENTION are further characterized as having selectivity against the PDE IV D, with the compound of example 2, for example, inhibiting PDE IV D at subnanomolar levels.

ANTI-INFLAMMATORY ACTIVITY

Inhibition of eosinophil activation

Blood samples (50ml) are collected from non-atopic laboratory staff with eosinophil numbers ranging between 0.06 and 0.47 x 10^9 L^{-1} (Microcellcounter F300, Sysmex). Venous blood is collected into centrifuge tubes containing 5 ml trisodium citrate (3.8%, pH 7.4, Sigma).

The anticoagulated blood is diluted (1:1, v:v) with phosphate-buffered saline (PBS, containing neither calcium nor magnesium, Gibco) and is layered onto 15 ml isotonic Percoll (density 1.082 - 1.085 g/ml, pH 7.4, Pharmacia), in a 50ml centrifuge tube (Falcon). Following centrifugation (30 min, 1000 x g, 20°C), mononuclear cells at the plasma/Percoll interface are aspirated carefully and discarded.

The neutrophil/eosinophil/erythrocyte pellet (circa 5 ml by volume) is gently resuspended in 35 ml of isotonic ammonium chloride solution (NH_4Cl, 155mM; $KHCO_3$, 10mM; EDTA, 0.1mM; 0-4°C). After 15 min, cells are washed twice (10 min, 400 x g, 4°C) in PBS containing foetal calf serum (2%, FCS, Gibco).

A magnetic cell separation system (Miltenyi Biotec) is used to separate eosinophils and neutrophils. This system is developed to separate cells in suspension according to surface markers, and comprises a permanent magnet, into which is placed a column that includes a magnetizable steel matrix. Prior to use, the column was equilibrated with PBS/FCS for 1 hr and then flushed with ice-cold PBS/FCS retrogradely via a 20ml syringe. A 21G hypodermic needle is attached to the base of the column and 1-2 mls of ice cold buffer allowed to efflux through the needle.

Following centrifugation of granulocytes, supernatant is aspirated and cells are gently resuspended with 100µl magnetic particles (anti-CD16 monoclonal antibody, conjugated to superparamagnetic particles by Miltenyi Biotec, Germany). The eosinophil/neutrophil/ anti CD16 magnetic particle mixture is incubated on ice for 40 min and then diluted to 5 ml with ice-cold PBS/FCS. The cell suspension is slowly introduced into the top of the column and the tap opened to allow the cells to move slowly into the steel matrix. The column is then washed with PBS/FCS (35ml), which is carefully added to the top of the

column so as not to disturb the magnetically labelled neutrophils already trapped in the steel matrix. Non-labelled eosinophils are collected in a 50ml centrifuge tube and washed (10 min, 400 x g, 4°C). The resulting pellet is resuspended in 5 ml Hank's balanced salt solution (HBSS) so that cell numbers and purity can be assessed prior to use. The separation column is removed from the magnet and the neutrophil fraction eluted. The column is then washed with PBS (50ml) and ethanol (absolute), and stored at 4°C.

Total cells are counted with a microcellcounter (Sysmex). One drop of lysing solution (Sysmex) is added to the sample, which after 30s is recounted to assess contamination with erythrocytes. Cytospin smears are prepared on a Shandon Cytospin 2 cytospinner (100 µl samples, 3 min, 500 rpm). These preparations are stained (Diff, Quick, Baxter) and differential cell counts determined by light microscopy, examining at least 500 cells. Cell viability is assessed by exclusion of trypan blue.

Eosinophils are diluted in HBSS and pipetted into 96 well microtitreplates (MTP) at 1-10 x 10^3 cells/well. Each well contains a 200 µl sample comprising:

100 µl eosinophil suspension
50 µl HBSS
10 µl lucigenin
20 µl activation stimulus
20 µl test compound

The samples are incubated with test compound or vehicle for 10 min prior to addition of an activation stimulus fMLP (10 µM) dissolved in dimethylsulphoxide and thereafter diluted in buffer, such that the highest solvent concentration used is 1% (at 100 µM test compound). MTPs are agitated (Titertek MTP mixer, Flow) to facilitate mixing of the cells and medium, and the MTP placed into a Hamamatsu luminometer. Total chemiluminescence and the temporal profile of each well is measured simultaneously over 20 min and the results expressed as arbitrary units, or as a percentage of fMLP-induced

chemiluminescence in the absence of test compound. Results are fitted to the Hill equation and IC_{50} values calculated automatically.

AGENTS OF THE INVENTION are active in the above test method at concentrations of the order of from 0.0001 to 0.5 μM, generally on the order of about 1 nM for the exemplified compounds. For example, the compound of example 2 has an IC_{50} in this assay of 1.9 nM, while the compound of example 4 has an IC_{50} of 0.71 nM.

BRONCHODILATOR ACTIVITY

Relaxation of human bronchus

Samples of human lungs dissected during surgery for cancer are obtained within 3 days after removal. Small bronchi (inner diameter ≈ 2 to 5 mm) are excised, cut into segments and placed in 2 ml Liquid Nitrogen Storage Ampoules filled with foetal calf serum (FCS) containing 1.8 M dimethyl sulphoxide (DMSO) and 0.1 M sucrose as cryoprotecting agents. The ampoules are placed in a polystyrol box (11x11x22cm) and slowly frozen at a mean cooling rate of about 0.6° C/min in a freezer maintained at -70°C. After 3-15 h the ampoules are transferred into liquid nitrogen (-196°C) where they are stored until use. Before use the tissues are exposed for 30-60 min to -70°C before being thawed within 2.5 min by placing the ampoules in a 37°C water bath. Thereafter the bronchial segments are rinsed by placing in a dish containing Krebs-Henseleit solution (composition mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03) at 37°C, cut into rings and suspended in 10 ml organ baths for isometric tension recording under a preload of about 1g. Concentration-response curves are produced by cumulative additions, each concentration being added when the maximum effect has been produced by the previous concentration. Papaverine (300 μM) is added at the end of the concentration-response curve to induce complete relaxation of the bronchial rings. This effect is taken as 100% relaxation.

In the above test model AGENTS OF THE INVENTION produce concentration-related relaxation of human bronchus ring preparations at concentrations of from 0.001 to 1.0 μM.

Suppression of bombesin induced bronchoconstriction

Male Dunkin-Hartley guinea pigs (400-800g) having free access to food and water prior to the experiment, are anaesthetized with sodium phenobarbital (100 mg/kg i.p.) and sodium pentobarbital (30 mg/kg i.p.), then paralyzed with gallamine (10 mg/kg i.m.). Animals, maintained at 37°C with a heated pad, controlled by a rectal thermometer, are ventilated via a tracheal cannula (about 8 ml/kg, 1 Hz) with a mixture of air and oxygen (45:55 v/v). Ventilation is monitored at the trachea by a pneumotachograph (Fleisch, type 0000) connected to a differential pressure transducer (MP 4514871, Validyne, USA) in line with the respiratory pump. Pressure changes within the thorax are monitored directly via an intrathoracic cannula, using a differential pressure transducer (MP 4524, Validyne) so that the pressure difference between the trachea and thorax can be measured and displayed. From these measurements of air-flow and transpulmonary pressure, both airway resistance (R_l, $cmH_2O/l/s$) and compliance (C_{dyn}) are calculated with a digital electronic respiratory analyzer (PMS 300, Mumed Ltd., UK) for each respiratory cycle. Blood pressure and heart rate are recorded from the carotid artery using a pressure transducer (P23Dd, Gould, USA).

When values for basal resistance and compliance are stable, sustained bronchoconstriction is induced by a continuous intravenous infusion of bombesin (100 ng/kg/min). Bombesin is dissolved in 100% ethanol and diluted with phosphate buffered saline.

Test compounds are administered when the response to bombesin was maximal and stable (ca. 2 min after start of bombesin infusion). Reversal of bronchoconstriction is assessed over 1 hour following either intratracheal or intraduodenal instillation or intravenous bolus injection. Bronchospasmolytic activity is expressed as % inhibition of the initial, maximal resistance (R_L) following the infusion of bombesin. ED_{50} values are given which represent

the dose which caused 50% reduction of the increase in resistance induced by bombesin. Duration of action is defined as the time in min where bronchoconstriction is reduced by 50% or more. Effects on blood pressure (BP) and heart rate (HR) are characterized by ED_{20} values; i.e. the doses which reduced BP or HR by 20% (5 min after administration).

Test compounds are administered either as solutions or, in the case of intratracheal or intraduodenal instillation, also as aqueous suspensions containing 0.5 % tragacanth in case of insoluble compounds. Suspensions are sonicated for 5 min to achieve a small particle size prior to administration.

Each drug is tested in 2 to 4 doses (n = 3-4 per dose). An adequate number of controls (5-6) is used.

In the above test model AGENTS OF THE INVENTION exhibit marked bronchodilator activity at dosages of the order of from 0.001 to 0.1 mg/kg i.v. or 0.1 to 5.0mg/kg i.d..

In accordance with the foregoing AGENTS OF THE INVENTION are useful for the treatment of inflammatory or obstructive airways disease or other conditions involving airways obstruction. In particular they are useful for the treatment of bronchial asthma.

Having regard to their anti-inflammatory activity, their influence on airways hyperreactivity and their profile in relation to PDE isoenzyme inhibition, in particular as selective type IV inhibitors, AGENTS OF THE INVENTION are useful for the treatment, in particular prophylactic treatment, of obstructive or inflammatory airways disease. Thus by continued and regular administration over prolonged periods of time AGENTS OF THE INVENTION are useful in providing advance protection against recurrence of bronchoconstrictor or other symptomatic attack consequential to obstructive or inflammatory airways disease or for the control, amelioration or reversal of basal status of such disease.

Having regard to their bronchodilator activity AGENTS OF THE INVENTION are useful as bronchodilators, e.g. for the treatment of chronic or acute broncho-constriction, e.g. for the symptomatic treatment of obstructive or inflammatory airways disease.

The words "treatment" and "treating" as used throughout the present specification and claims in relation to obstructive or inflammatory airways disease are to be understood accordingly as embracing both prophylactic and symptomatic modes of therapy.

In accordance with the foregoing the present invention further provides

A. A method

a) for the treatment of airways hyperreactivity,

b) of effecting bronchodilation or, in particular,

c) of treating obstructive or inflammatory airways disease, especially asthma,

in a subject in need thereof, which method comprises administering to said subject an effective amount of an AGENT OF THE INVENTION.

Obstructive or inflammatory airways diseases to which the present invention applies include asthma, pneumoconiosis, chronic obstructive airways or pulmonary disease (COAD or COPD) and adult respiratory distress syndrome (ARDS), as well as exacerbation of airways hyperreactivity consequent to other drug therapy, e.g. aspirin or β-agonist therapy.

The present invention is applicable to the treatment of asthma of whatever type or genesis, including intrinsic and, especially, extrinsic asthma. It is applicable to the treatment of allergic (atopic/IgE-mediated) asthma. It is also applicable to the treatment of non-atopic asthma, including e.g. bronchitic, exercise induced and occupational asthma, asthma induced following bacterial infection and other non-allergic asthmas. It is further applicable to the treatment of wheezy infant syndrome (infant, incipient asthma).

The invention is applicable to the treatment of pneumoconiosis of whatever type or genesis including, for example, aluminosis, anthracosis, asbestosis, chalicosis, ptilosis, siderosis, silicosis, tobacoosis and byssinosis.

The invention is applicable to the treatment of COPD or COAD including chronic bronchitis, pulmonary emphysaema or dyspnea associated therewith.

The invention is also applicable to the treatment of bronchitis of whatever type or genesis including, e.g. acute, arachidic, catarrhal, chronic, croupus or phthinoid bronchitis etc..

Having regard to their activity as selective inhibitors of TNF-α release, AGENTS OF THE INVENTION are also useful for the down-regulation or inhibition of TNF-α release, e.g. for the treatment of diseases or conditions in which TNF-α release is implicated or plays a mediating role, e.g. diseases or conditions having an aetiology involving or comprising morbid, for example undesirable, excessive or unregulated TNF-α release, in particular for the treatment of cachexia or endotoxin shock and in treatment of AIDS.

The method of the invention is applicable to the treatment of cachexia associated with morbid TNF-α release or TNF-α blood-serum levels of whatever origin, including cachexia consequential to, e.g. bacterial, viral or parasitic, infection or to deprivation or deterioration of humoral or other organic, e.g. renal function. It is for example applicable to the treatment of cancerous, malarial and vermal cachexia, cachexia resulting from dysfunction of the pituitary, thyroid or thymus glands as well as uremic cachexia. It is in particular applicable to the treatment of AIDS-related cachexia, i.e. cachexia consequential to or associated with to HIV infection.

The method of the invention is also applicable to the treatment of septic shock, e.g., shock conditions resulting from bacterial infection. In this regard it is to be noted that the present invention provides a method for the treatment of septic shock as such as well as of conditions consequential to or symptomatic of septic or shock, for example ARDS (adult respiratory distress syndrome).

The method of the invention is further applicable to the treatment of disease consequential to HIV infection, e.g. AIDS, e.g. to the amelioration or control of the advance of such disease.

Having regard to their profile in relation to inhibition of PDE isoenzymes and/or TNFα release inhibition, as well as their immunosuppressive activity, AGENTS OF THE INVENTION are also useful as immunosuppressive agents, e.g. for the treatment of autoimmune diseases, in particular for the treatment of autoimmune diseases in which inflammatory processes are implicated or which have an inflammatory component or aetiology, or as anti-inflammatory agents for the treatment of inflammatory disease in particular for the treatment of inflammatory disease in which autoimmune reactions are implicated or having an autoimmune component or aetiology.

Examples of such disease to which the present invention is applicable include autoimmune hematological disorders (e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, polychondritis, scleroderma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (e.g. ulcerative colitis and Crohn's disease) endocrine ophthalmopathy, Grave's disease, sarcoidosis, alveolitis, chronic hypersensitivity pneumonitis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis and glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy), as well as inflammatory and/or hyperproliferative skin diseases such as psoriasis atopic dermatitis, pemphigus and, in particular, contact dermatitis, e.g. allergic contact dermatitis.

AGENTS OF THE INVENTION are in particular useful for the treatment of arthritis, and other rheumatic or inflammatory disease, especially for the treatment of rheumatoid arthritis.

As immunosuppressants AGENTS OF THE INVENTION are further indicated for use in the prevention of graft rejection, e.g. for the maintenance of allogenic organ transplants or the like, e.g. in relation to kidney, liver, lung, heart, heart-lung, bowel, bone-marrow, skin, or corneal transplant.

Having regard to their anti-inflammatory activity, in particular in relation to inhibition of eosinophil activation, AGENTS OF THE INVENTION are also useful for the treatment of eosinophil related disorders, e.g. eosinophilia, in particular eosinophil related disorders of the airways (e.g. involving morbid eosinophilic infiltration of pulmonary tissues) including hypereosinophilia as it effects the airways and/or lungs as well as, for example, eosinophil-related disorders of the airways consequential or concomitant to Löffler's syndrome, eosinophilic pneumonia, parasitic (in particular metazoal) infestation (including tropical eosinophilia), bronchopulmonary aspergillosis, polyarteritis nodosa (including Churg-Strauss syndrome), eosinophilic granuloma and eosinophil-related disorders affecting the airways occasioned by drug-reaction.

Having regard to their ability to interact synergistically with immunosuppressive and/or anti-inflammatory drug substances, AGENTS OF THE INVENTION are also useful as co-therapeutic agents for use in conjunction with such drugs, e.g. as potentiators of therapeutic activity of such drugs or as means of reducing required dosaging or potential side effects of such drugs. Drug substances with which AGENTS OF THE INVENTION may suitably be co-administered include, e.g. cyclopeptide, cyclopeptolide or macrolide immunosuppressive or anti-inflammatory drug substances, for examples drugs belonging to the cyclosporin class, e.g. cyclosporins A or G, the drug substances tacrolimus (also known as FK 506), ascomycin and rapamycin and their various known congeners and derivatives, as well as glucocorticosteroid drugs. Diseases to which such co-therapy may be applied include e.g. any disease or condition requiring immunosuppressive or anti-inflammatory drug therapy, e.g. as hereinbefore set forth. In particular AGENTS OF THE INVENTION are suitable for use in co-therapy as aforesaid, e.g. for the purposes of immunosuppressive, anti-inflammatory or anti-asthmatic treatment, e.g. to achieve cyclosporin, e.g. cyclosporin A-, macrolide- or steroid-sparing effect.

Having regard to their profile in relation to inhibition of PDE isoenzymes, in particular their profile as selective type IV inhibitors, AGENTS OF THE INVENTION are further useful as type IV PDE inhibitors, for example for the treatment of disease involving tissue calcium depletion, in particular degenerative diseases of the bone and joint involving calcium depletion, especially osteoporosis. In this regard they are further useful for the treatment of allergic inflammatory diseases such as rhinitis, conjunctivitis, atopic dermatitis, urticaria and gastro-intestinal allergies; as vasodilators, e.g. for the treatment of angina, hypertension, congestive heart failure and multi-infarct dementia; and for the treatment of other conditions where inhibition of PDE IV is indicated, for example, depression, conditions and diseases characterized by impaired cognitive function including Alzheimer's disease, Parkinson's disease and stroke.

CNS ACTIVITY

The neuropathology of Parkinson's disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra. Elevation of cAMP levels in dopaminergic neurons improves their survival in culture and protects them from the effects of neurotoxic agents. In vivo, the Type IV phosphodiesterase inhibitors of the invention reduce MPTP-induced dopamine depletion in the striatum of C57BL/6 mice. Furthermore, the reduction in the number of tyrosine-hydroxylase-positive neurons caused by the toxin is partly prevented by the inhibitors.

Cell culture, dopamine or MPP^+ uptake and survival of dopaminergic neurons

Primary cultures containing dopaminergic neurons are prepared from E14 fetal rat ventral mesencephalon and dopamine or MPP+ uptake measured by the method described in Hartikka et al., J. Neurosci. Res., 32, 190-201, 1992. A 15 mm culture well usually contains 6×10^5 neurons. Dopamine or MPP^+ uptake is measured using tritiated dopamine or MPP^+ at concentrations of 50 nM (spec. act. 45 Ci/mmol, New England Nuclear) or 1 µM (spec. activity 80 Ci/mmol, New England Nuclear), respectively. The survival of

dopaminergic neurons in the culture is assayed by counting tyrosine hydroxylase positive (TH^+) neurons previously stained with a mouse anti-TH antibody (Boehringer Mannheim).

MPTP-injected mice

C57BL/6 mice are subcutaneously injected with MPTP or saline. A second and in some experiments third injection of MPTP follows two and four hours later. At 0, 2, and 4 hours, are orally dosed with test compound in saline or same volume of saline alone. Seven days later the animals are sacrificed by decapitation and the monoamines are determined by HPLC [Schneider et al., Science 256, 843-846 (1992)] or the brains processed for tyrosine hydroxylase (TH) immunocytochemistry. For this the mice are intracardially perfused with 4% paraformaldehyde and whole brains further fixed in cold 4% paraformaldehyde overnight. Cryosections (40 µm) are cut in the midbrain region, blocked for endogenous peroxidases with 0.3% peroxide in methanol for 15 minutes and digested with 0.1% trypsin in PBS for 10 minutes.

This is followed by a 48-hour incubation at 4°C with a polyclonal rabbit antiserum against TH (Eugene Tech. International Inc., New Jersey) diluted 1:50 in PBS with 2% goat serum and 0.2% Triton-X. Sections are further developed using the Vectastain Elite ABC Kit (Vector Laboratories, CA) and nickel-diaminobenzidine (DAB) enhancement. Prior to the DAB reaction, sections are incubated for 10 minutes in 2% $NiSO_4$ in 0.1 M Tris-HCl pH 7.4. The solution is tipped off and replaced with 0.05% DAB, 0.2% $NiSO_4$, 0.3% H_2O_2 in 0.1 M Tris.

AGENTS OF THE INVENTION are active in this assay. For example, MPTP-injected mice receiving the compound of example 2 at a dosage of 4 mg/kg p.o. at 0, 2, and 4 hours show dopamine levels 25% higher than those seen in untreated controls. Therefore AGENTS OF THE INVENTION are useful to inhibit dopamine depletion and enhance dopamine levels, and are thus useful, e.g., in the treatment of Parkinson's disease.

Furthermore AGENTS OF THE INVENTION promote the survival of noradrenergic as well as serotoninergic neurons in culture. Therefore AGENTS OF THE INVENTION are useful in the treatment of neurodegenerative diseases.

Such neurodegenerative diseases include primary degenerative dementia, e.g. senile dementia, particularly senile dementia of the Alzheimer type, as well as senile mental decline, e.g. senile cognitive decline, and confusional conditions in the elderly.

Furthermore, AGENTS OF THE INVENTION improve the survival of motor neurons and sensory neurons in culture and suppress the expression of Tumor Necrosis Factors.

Therefore AGENTS OF THE INVENTION are useful in the treatment of multiple sclerosis, amyotrophic lateral sclerosis and other motor neuron or autoimmune diseases.

Moreover after injection of AGENTS OF THE INVENTION into mice, DOPAC/dopamine as well as 5-HIAA/serotonin ratios in the striatum are elevated by a factor of 2 and 1.5 respectively. Therefore AGENTS OF THE INVENTION are useful in the treatment of depression.

In accordance with the foregoing the present invention also provides:

B. A method

a) for the down-regulation or inhibition of TNF-α release,

b) for the inhibition of PDE IV isoenzyme activity,

c) of effecting immunosuppression,

d) for the treatment of inflammatory or autoimmune diseases or conditions,

e) for the treatment of depression or conditions and diseases characterized by impaired cognitive function including Alzheimer's disease, Parkinson's disease and stroke, or

e) for the treatment of any particular condition or disease as hereinabove set forth,

in a subject in need thereof, which method comprises administering to said subject an effective amount of an AGENT OF THE INVENTION.

The present invention also provides:

C. An AGENT OF THE INVENTION for use as a pharmaceutical, for example for use in any method or in the treatment of any disease or condition as hereinbefore set forth, e.g. as defined under A or B above, and

D. A pharmaceutical composition comprising an AGENT OF THE INVENTION, optionally in combination or association with a pharmaceutically acceptable diluent or carrier therefor, e.g. for use in any method as hereinbefore set forth, e.g., as defined under A or B above, for example a pharmaceutical composition in inhalable form or in oral dosage form.

Dosages employed in practicing the present invention will of course vary depending, e.g. on the particular disease or condition to be treated, the particular agent of the invention used, the mode of administration and the therapy desired. In general, however, satisfactory results, e.g. for the treatment of obstructive or inflammatory disease, e.g. for asthma therapy are indicated to be obtained at dosages of the order from about 0.01 to 2.0 mg/kg/p.o.. In larger mammals, for example humans, an indicated daily dosage for oral administration, for treatment of airways hyperreactivity or for treatment of inflammatory events in obstructive or inflammatory airways disease will be in the range of from about 0.75 to 150 mg, conveniently administered 1 x or in divided doses 2 to 4x daily or in sustained release form. Unit dosage forms for oral administration thus suitably comprise from about 0.2 to 75 or 150, e.g. from about 0.2 or 2.0 to 50, 75 or 100 mg AGENTS OF THE INVENTION, together with a pharmaceutically acceptable diluent or carrier therefor.

For use in the treatment of chronic or obstructive airways disease, e.g. asthma AGENTS OF THE INVENTION may also be administered by the inhaled route. Again dosages employed will vary, e.g. depending on the particular disease or condition, the particular

AGENT OF THE INVENTION employed, the particular mode of administration (e.g. whether by dry powder inhalation or otherwise) and the effect desired. In general, however, an indicated inhaled daily dosage will be of the order of from about 2.5 to about 130.0 μg/kg/day e.g. from about 13.0 to about 60.0 μg/kg/day. For larger mammals, for example humans, an indicated daily dosage for administration by inhalation, e.g. in the treatment of asthma, will be in the range of from about 0.2 to about 10.0 mg, e.g. from about 1 to about 5 mg, conveniently given in one single administration or 2 or 3 separate administrations throughout the day. An appropriate dosage per administration will thus be of the order of from about 200 μg to about 3.3 mg, with administration up to 3 times daily, suitably administered from a dry powder inhalation delivery device in a series of 2 to 8 puffs at each administration.

Similarly satisfactory results for the treatment of Parkinson's disease and other neurodegenerative diseases, motor neuron or autoimmune diseases, such as those indicated above, and of depression, are indicated to be obtained at dosages of the order from about 0.5 to 5.0 mg/kg p.o.. In larger mammals, for example humans, an indicated daily dosage for oral administration, for treatment of said neurodegenerative diseases and depression will be in the range of from about 40 to 400 mg, conveniently administered 1 x or in divided doses 2 to 4x daily or in sustained release form. Unit dosage forms for oral administration thus suitably comprise from about 10 to 200 or 400 mg AGENTS OF THE INVENTION, together with a pharmaceutically acceptable diluent or carrier therefor.

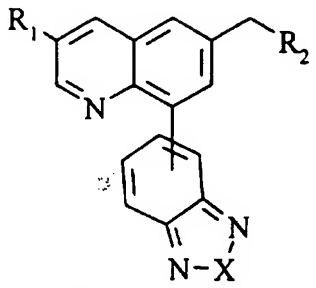
AGENTS OF THE INVENTION may also be administered by any other appropriate route, e.g. by infusion, for example for the treatment of septic shock; nasally, for example for the treatment of rhinitis; ocularly, for example for the treatment of autoimmune diseases of the eye; dermally, i.e. topically to the skin, for example for the treatment of dermatoses or psoriasis; or rectally, e.g. via enemation or suppository, for example for the treatment of inflammatory bowel disease. Suitable dosages for application by such routes will generally be lower, e.g., of the order of 10 to 100x less, than those required for oral administration.

Pharmaceutical compositions comprising AGENTS OF THE INVENTION may be prepared using conventional diluents or excipients as known in the art and employing any of the techniques known in the galenic art. Thus oral dosage forms may include tablets, capsules and the like. Compositions for inhalation may comprise aerosol or other atomisable formulations as well as inhalable dry powder formulations, with or without diluent, for administration by any appropriate dry powder inhalation system as known in the art. Formulations for dermal administration may take the form of creams, ointments, gels, or transdermal delivery systems, e.g. patches and, in addition to inert diluents or carriers, may suitably contain skin penetration enhancing agents, again as known in the art.

CLAIMS

1. An 8-(benzo[*c*]thiadiazolyl, benzo[*c*]furazanyl, or 2-methyl-2H-benzo[*d*]triazolyl)-quinoline, in free or N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

2. A compound according to claim 1 of formula I:



Formula I

wherein

X is -O-, -S-, or $-N(CH_3)-$,

R_1 is hydrogen or (C_{1-4})alkyl, and

R_2 is (C_{1-4})alkyl or pyridyl

in free or N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

3. A compound according to claim 1 or 2 selected from

i) 8-(benzo[*c*]furazan-4-yl)-6-(pyridin-4-yl)-quinoline

ii) 8-(benzo[*c*]furazan-4-yl)-3-methyl-6-(pyridin-4-yl)-quinoline

iii) 8-(benzo[*c*]furazan-3-yl)-6-(pyridin-4-yl-methyl)-quinoline

iv) 8-(benzo[*c*]thiadiazol-4-yl)-6-(pyridin-4-yl)-quinoline

v) 8-(benzo[*c*]thiadiazol-4-yl)-3-methyl-6-(pyridin-4-yl)-quinoline

vi) 8-(2-methyl-2H-benzo[*d*]triazol-5-yl)-6-(pyridin-4-ylmethyl)-quinoline

in free or N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

4. A compound according to claim 1, 2, or 3 for use as a pharmaceutical.

5. A pharmaceutical composition comprising a compound of claim 1, 2 or 3, optionally in association with a pharmaceutically acceptable carrier or diluent.

6. The use of a compound according to any of claims 1, 2, or 3, in the manufacture of a medicament for the treatment of

a) obstructive or inflammatory airways disease

b) inflammatory or autoimmune diseases or conditions,

e) depression, Alzheimer's disease, Parkinson's disease or stroke.

7. A method of treating

a) obstructive or inflammatory airways disease

b) inflammatory or autoimmune diseases or conditions,

e) depression, Alzheimer's disease, Parkinson's disease or stroke

comprising administering an effective amount of a compound according to claim 1, 2, or 3 to a patient in need thereof.

8. A process for the production of a compound according to claim 1, 2, or 3 comprising the steps of reacting

(i) a 3-Q- or 4-Q- -benzo[*c*]thiadiazole, -benzo[*c*]furazan, or -2-methyl-2H-benzo[*d*]triazole with an 8-Q'-quinoline

wherein Q and Q' are leaving groups capable of participating in a cross-coupling reaction,

(ii) optionally N-alkylating or N-ozidizing the product,

and recovering the compound thus obtained, in free, N-oxide or pharmaceutically acceptable acid addition or ammonium salt form.

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/EP 96/04978

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D413/14 A61K31/41 C07D417/14 C07D401/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 22852 A (SYNTEX (U.S.A.) INC.) 13 October 1994 see page 2 - page 3 ---	1,2,6
A	EP 0 490 823 A (SANDOZ LTD.) 17 June 1992 see page 7; claims -----	1,2,6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search
27 January 1997

Date of mailing of the international search report
06.02.97

Name and mailing address of the ISA
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 96/04978

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 7 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/EP 96/04978

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9422852	13-10-94	US-A-	5455252	03-10-95
		AU-A-	6412994	24-10-94
		CA-A-	2159603	13-10-94
		EP-A-	0691966	17-01-96
		FI-A-	954651	29-09-95
		HU-A-	9500111	28-06-95
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		NO-A-	953879	22-11-95
EP-A-490823	17-06-92	AT-T-	145201	15-11-96
		AU-B-	643575	18-11-93
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		CA-A-	2057524	14-06-92
		DE-D-	69123124	19-12-96
		ES-T-	2093694	01-01-97
		IL-A-	100328	08-12-95
		JP-A-	4275276	30-09-92
		JP-B-	7064820	12-07-95
		PL-B-	168329	29-02-96
		RU-C-	2060992	27-05-96
		US-A-	5177085	05-01-93
		ZA-A-	9109858	14-06-93